NUCLEOTIDE ANALOGS BASED ON PENTAERYTHRITOL - AN HYPOTHESIS

ALAN W. SCHWARTZ

Laboratory for Evolutionary Biology, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

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Abstract. The synthesis of ribose and ribose-based nucleotides under reasonable prebiotic conditions has not been achieved. Glycerol has been suggested as a structural unit that might have preceded ribose in the evolutionary emergence of RNA. Template-directed oligomerizations of nucleotide analogs based on glycerol, however, have been only partially successful. Recent studies on the effect of ultraviolet irradiation of formaldehyde solutions have shown that the reduced sugar pentaerythritol is formed with great specificity. I argue that pentaerythritol is potentially capable of being converted by simple chemistry into a series of nucleoside analogs related to barbituric acid. These analogs may be able to take part in nucleic acid-like interactions and could therefore be of potential interest as a new class of candidates as RNA precursors.

Background

During the past few years, the problem of the origin of the first self-replicating RNA molecules (Orgel, 1992) has become inextricably bound up with the question of the prebiotic synthesis of sugars. Reid and Orgel (1967) and, more recently, Shapiro (1986) have pointed out that the autocatalytic formose reaction results in such a complex mixture of carbohydrates that it is inconceivable that spontaneous synthesis of nucleotides and consequently of RNA could have been possible as a result of this process. We have recently reconfirmed and extended these conclusions (Schwartz and de Graaf, in press). Recent work in Eschenmoser's laboratory on the favored synthesis of ribose diphosphate (Müller *et al.*, 1990) and allose triphosphate (Eschenmoser and Dobler, 1992) by base-catalyzed condensations of glycolaldehyde-phosphate have added a new perspective to this problem, although the starting material must first be synthesized using classical methods and organic solvents. The seriousness of the problem which nucleotide synthesis presents for understanding the origin of RNA was accentuated by the demonstration that poly(C)-directed oligomerization of the activated nucleotide 2-MeImpG is inhibited if the monomer is racemic (Joyce *et al.*, 1984). If even this model template-directed reaction, carried out with structurally homogeneous reactants, is defeated by the phenomenon of chirality, how could any self-replicating oligonucleotide possibly arise out of a mixture of randomly formed purines, pyrimidines and sugars?

A theoretical solution to the problem was offered by Spach (1984) and by Joyce *et al.* (1987). It was suggested that the first self-replicating molecules might have been based on less demanding structures than polynucleotides.

Specifically *prochiral* nucleotide analogs derived from glycerol might have preceded

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the formation of the first ribonucleotides. Previous work from our laboratory has been based on this proposal. We have demonstrated that a pyrophosphate backbone can be substituted for a phosphodiester backbone without destroying the capability of carrying out template-directed oligomerization (Schwartz and Orgel, 1985; Schwartz *et al.*, 1987; Visscher and Schwartz 1988 and 1990; Visscher *et al.*, 1989). However, when both template and product oligomers are based on pyrophosphate-linked analogs, the reactions are much less efficient than the oligomerization of

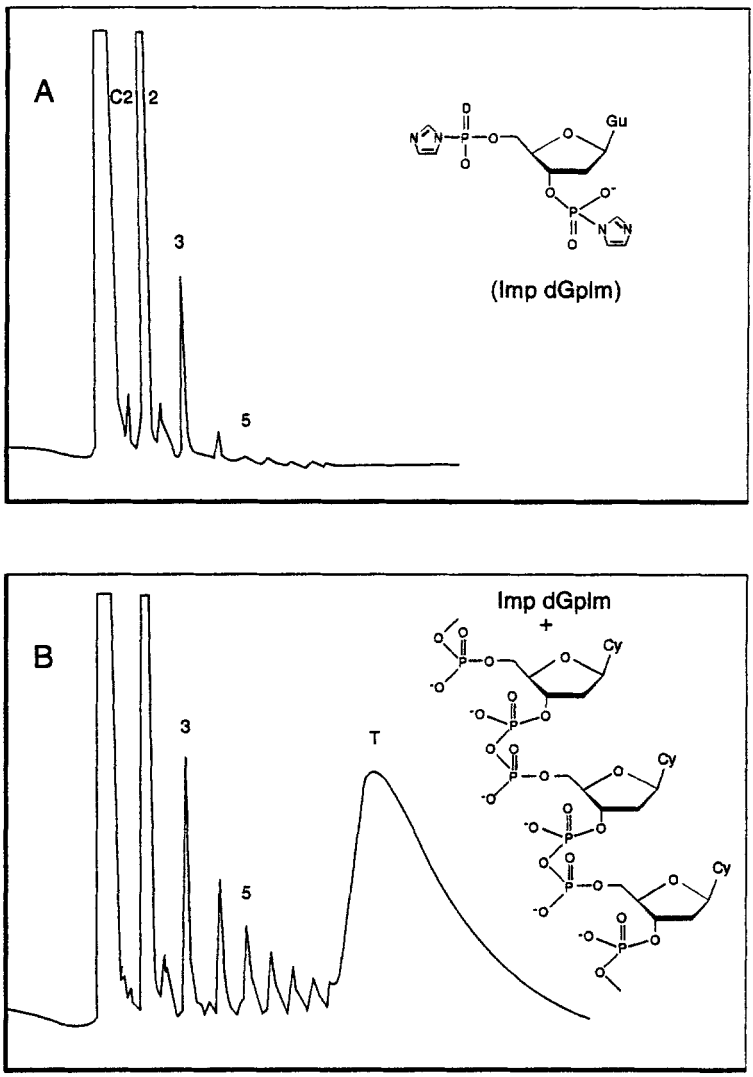


Figure 1. HPLC on RPC-5 of the products of the oligomerization of ImpdGpIm in the presence of a pyrophosphate-linked polynucleotide analog (Visscher *et al.*, 1989). The structures of the monomer and the resulting products are shown. A) ImpdGpIm alone, B) ImpdGpIm in the presence of the template (T).

2-MeImpG on poly(C) (Figures 1 and 2). In the case of prochiral glycerol-based analogs, the limited catalysis obtained in the presence of the template raises questions concerning the validity of this approach. Both the flexibility of these analogs, which leads to a considerable amount of cyclization of the monomer (Visscher and Schwartz, 1988), as well as the atactic configuration of the oligomers, may be factors which contribute to limiting the extent of the template-directed oligomerization (Visscher and Schwartz, 1990). Nevertheless, it should be realized that the degree of catalysis observed in the oligomerization of the glycerol-based monomer in the presence

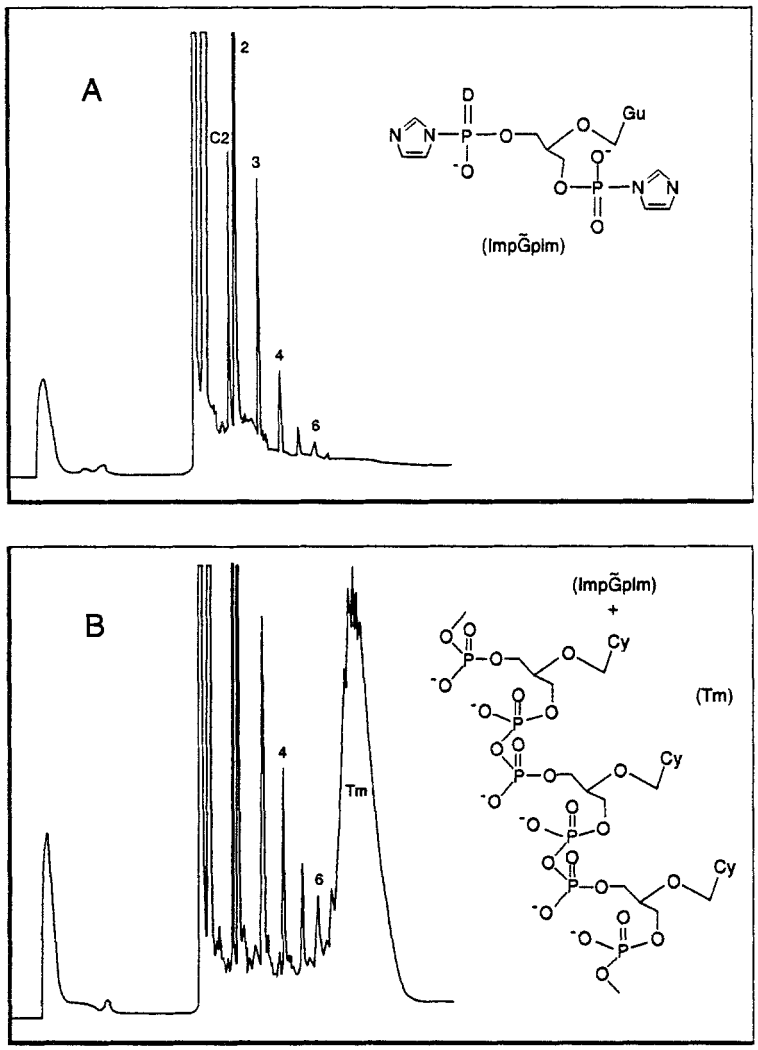


Figure 2. HPLC on RPC-5 of the products of the oligomerization of a glycerol-based analog in the presence of a pyrophosphate-linked, glycerol-based polynucleotide analog (Visscher and Schwartz, 1990). The structures of the monomer and the resulting products are shown. A) ImpG̃pIm alone, B)ImpG̃pIm in the presence of the template (Tm).

of the randomly polymerized glycerol-based template is probably superior to the results which would be expected from carrying out a similar polymerization of the corresponding deoxyribose-diphosphate. In the latter case, the template would not only consist of randomly assorted D- and L-units, but would contain mixtures of 3'-3', 3'-5' and 5'-5' linkages.

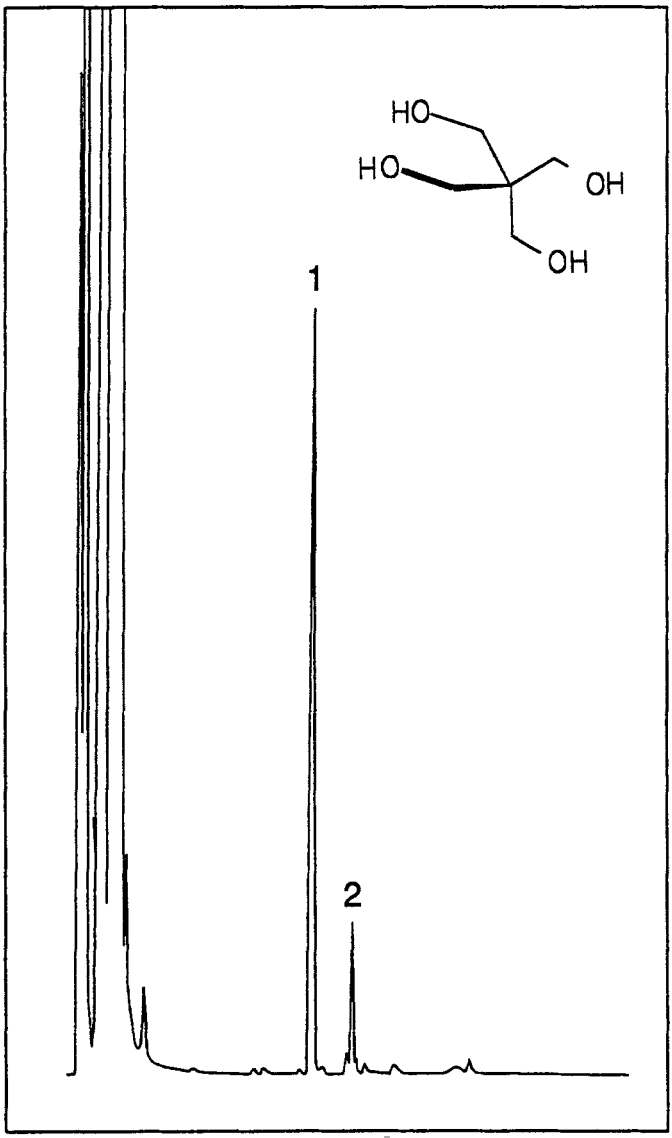
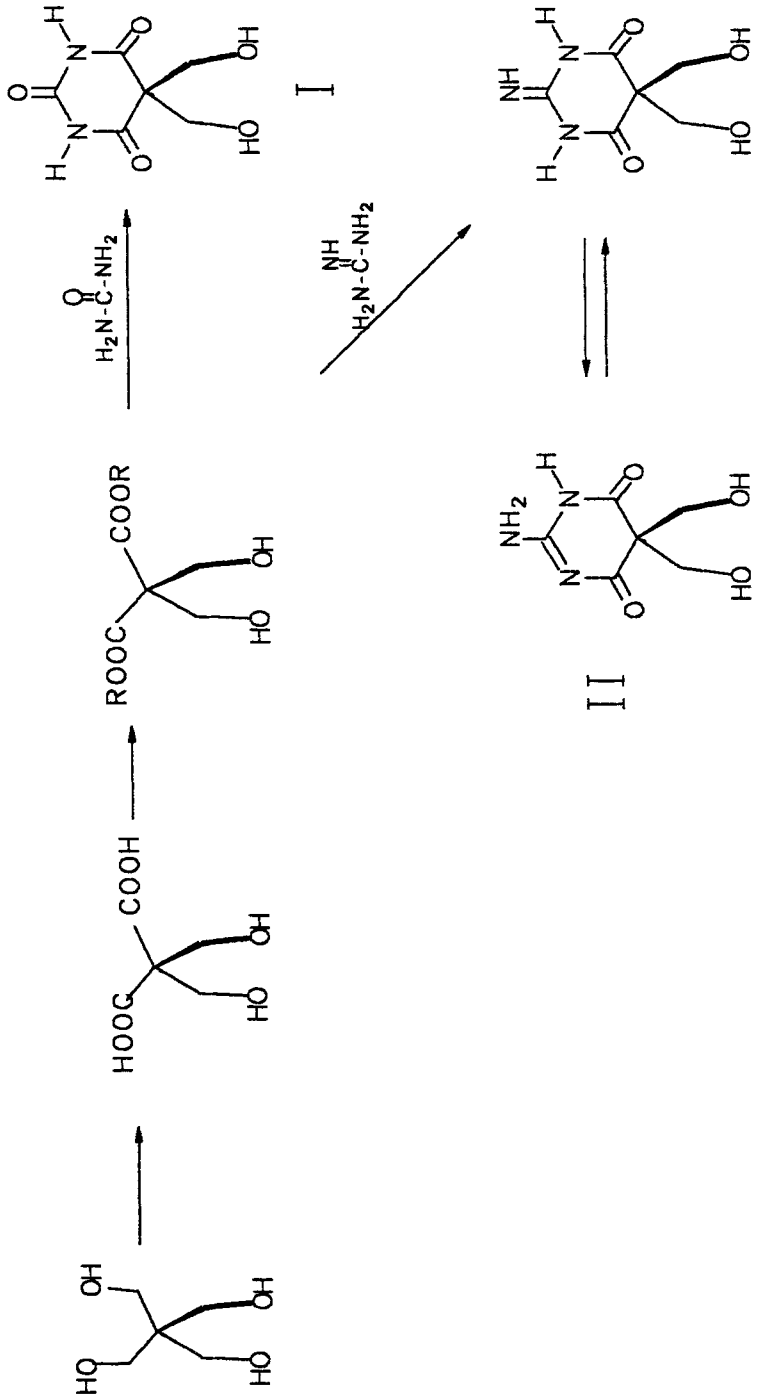


Figure 3. Gas-chromatographic analysis of the products of the ultraviolet irradiaction of 0.1 M formaldehyde in the presence of 0.05M K_2CO_3. Irradiation for 10 h, followed by 19 days at room temperature (Schwartz and de Graaf, in press). Peak 1 is pentaerythritol (stucture shown), peak 2 is an unidentified product.


I
II
COOR
ROOC
COOH
HOOC
OH
HO
NH
NH2
O
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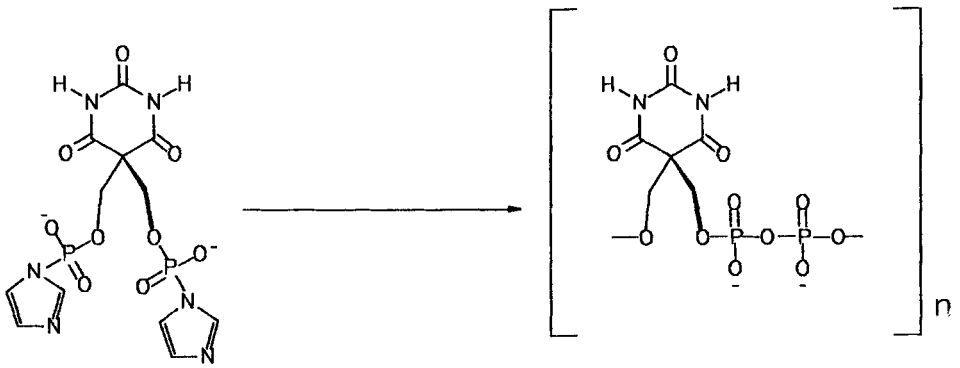


Figure 4. The oligomerization of the bisphosphoimidazolide of analog I.

Pentaerythritol-Based Analogs

In spite of the above-described interest in glycerol as a building block for nucleotide analogs, there has been no reasonably prebiotic synthesis described for the parent carbohydrates, glyceraldehyde and dihydroxyacetone. For these and other reasons, we recently reexamined the base-catalyzed condensation of formaldehyde under mild conditions. Although we confirmed earlier conclusions that the autocatalytic formose reaction is intrinsically nonselective (Schwartz and de Graaf, in press), and therefore not a plausible source of any individual sugar, we found that ultraviolet irradiation of 0.1 M formaldehyde at moderately alkaline pH values results in an unexpectedly efficient synthesis of the reduced carbohydrate pentaerythritol (Figure 3). The details of the mechanism of the formation of pentaerythritol are under investigation and will be reported upon elsewhere. However, this highly selective synthesis, taken together with the symmetrical structure of pentaerythritol, suggested the possibility that it might have played a role in some RNA precursor, similar to that which was postulated for glycerol. Analysis of the structure of pentaerythritol reveals that a small number of chemical procedures can produce a nonchiral nucleotide analog.

Scheme 1 shows that oxidation of any two OH functions produces the possibility of a ring closure via activation of the acid groups and condensation with urea. The resulting compound (I) is a pyrimidine nucleoside analog related to barbituric acid. Ring closure with guanidine would produce the analog II. In a second step, we can imagine the phosphorylation of the primary alcohol groups of these analogs and their activation and oligomerization to produce pyrophosphate-linked chains (Figure 4). Analogs I and II resemble uracil and adenine and theoretically could form an analogous set of hydrogen bonds (Figure 5). Self-complementary structures are also possible (Figure 6). Recent work on the formation of hydrogen-bonded complexes utilizing barbituric acid with diaminopyrimidine demonstrate the feasibility of this type of pair formation (Lehn *et al.*, 1990). Even a greater range of potential base pairs becomes possible if we consider the additional structures III–VI

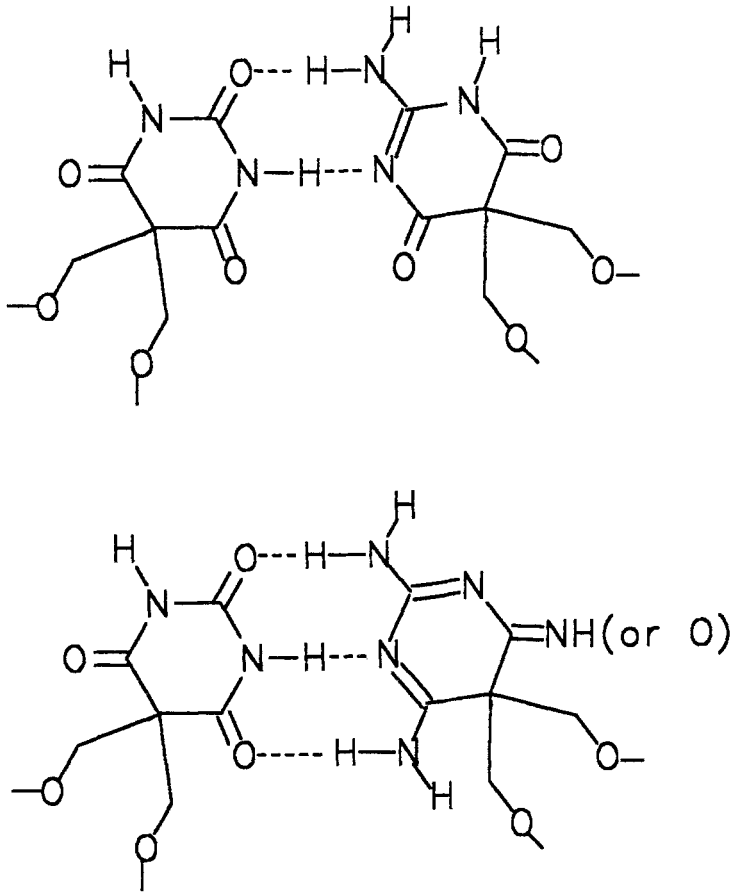


Figure 5. Possible pair formation for the analogs I and II (top) and I and IV (or VI, bottom).

(Figure 7). Although these compounds might not be derivable directly from pentaerythritol by simple aqueous chemistry, they suggest additional and intriguing possibilities. The triple-bonded complex shown between analogs I and IV (or VI, Figure 5) is one example of a possibly favorable interaction.

In all of the above cases, the plane of symmetry which lies in the heterocyclic ring would result in the production of stereochemically ordered oligomers. The possibility of template-directed oligomerization of complementary analogs seems sufficiently attractive to make synthesis of some of these compounds worthwhile. The extent to which particular pairs might form in aqueous solution is difficult to predict, as they could only be expected to be stable if stacking interactions were to assist in stablizing them. Additionally, self-complimentary structures would compete with complementary pair formation. Only synthesis and a study of the properties of these compounds will answer many of these questions. We expect therefore to put some of these suggestions to the test in the near future.

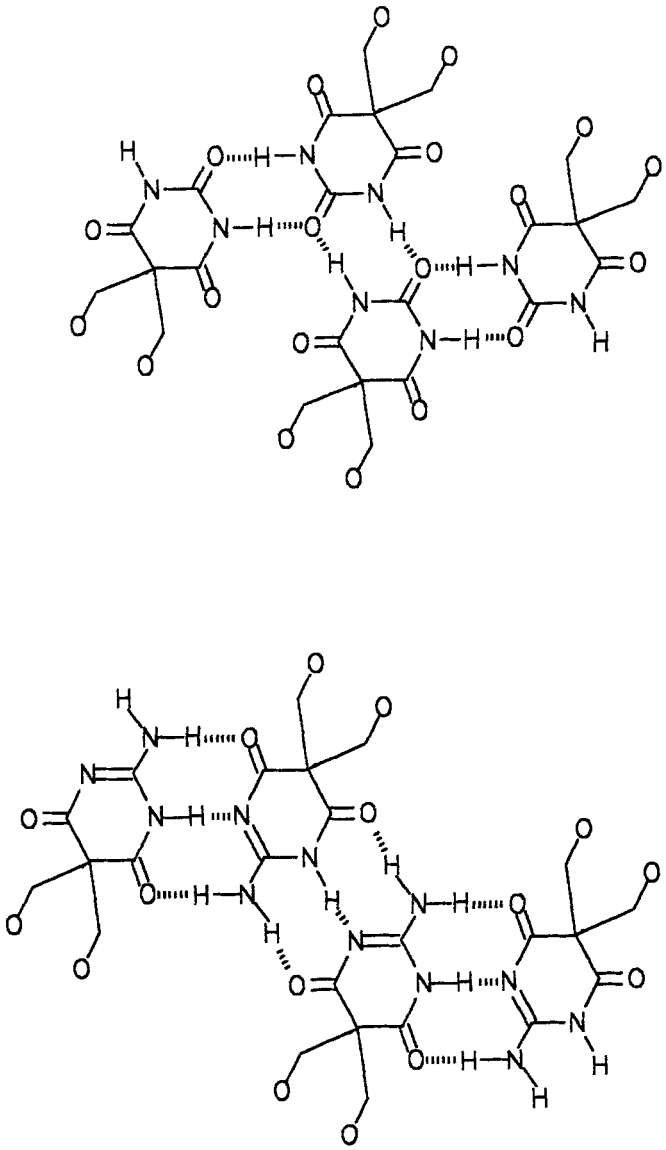
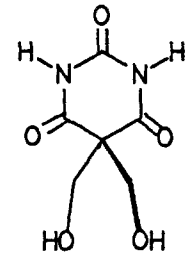
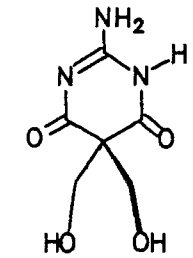


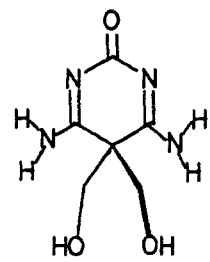
Figure 6. Possible self-complimentary structures which could be formed by the analogs I (top) and II (bottom).



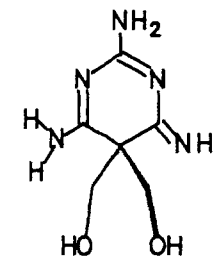
I



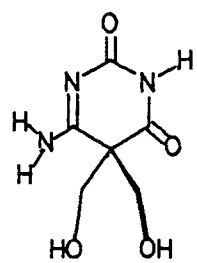
II



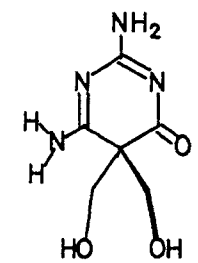
III



IV



V



VI

Figure 7. The structures of pentaerythritol-based analogs.

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